Sexual Hieracium pilosella plants are better inter-specific, while apomictic plants are better intra-specific competitors

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Abstract: Apomixis, asexual reproduction through seeds, occurs in over 40 plant families. This widespread phenomenon can lead to the fixation of successful genotypes, resulting in a fitness advantage. On the other hand, apomicts are expected to lose their fitness advantage if the environment changes because of their limited evolutionary potential, which is due to low genetic variability and the potential accumulation of deleterious somatic mutations. Nonetheless, some apomicts have been extremely successful, for example certain apomictic accessions of Hieracium pilosella L. from New Zealand, where the plant is invasive. Here, we investigate whether the success of these apomictic accessions could be due to a fitness advantage by comparing the vegetative competitiveness of apomictic H. pilosella from New Zealand with sexual accessions of H. pilosella from Europe. Sexual and apomictic plants were grown either (A) alone (no competition), (B) in competition with the other type (intra-specific competition), (C) in competition with the grass Bromus erectus (inter-specific competition), and (D) in competition with the other type and the grass B. erectus (intra- and inter-specific competition). To distinguish effects of apomixis and the region of origin, different H. pilosella lineages were compared. Furthermore, experiments were carried out to investigate effects of the ploidy level. We show that sexual plants are better inter-specific competitors than apomicts in terms of vegetative reproduction (number of stolons) and vegetative spread (stolon length), while apomicts do better than sexuals in intra-specific competition. The magnitude of the effect was in some cases dependent on the ploidy levels of the plants. Furthermore, apomicts always produced more stolons than sexuals, suggesting potential displacement of sexuals by apomicts where they co-occur.

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Sexual *Hieracium pilosella* plants are better between-species, while apomictic plants are better within-species competitors

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Abstract. Apomixis, asexual reproduction through seeds, is known in over 40 plant families. This widespread phenomenon can lead to the fixation of successful genotypes, resulting in a fitness advantage. On the other hand, apomicts are expected to lose their fitness advantage if the environment changes because of their limited evolutionary potential due to low genetic variability and because of the potential accumulation of deleterious somatic mutations over time. Nonetheless, some apomicts have been extremely successful, for example certain apomictic accessions of *Hieracium pilosella* L. from New Zealand, where the plant is invasive. Here, we investigate whether the success of these apomictic accessions could be due to a fitness advantage by comparing the vegetative competitiveness of apomictic *H. pilosella* from New Zealand with sexual accessions of *H. pilosella* from Europe. Sexual and apomictic plants were grown either (i) alone (no competition), (ii) in competition with the other type (intra-specific competition), or/and (iii/iv) in competition with the grass *Bromus erectus* (inter-specific competition/intra- and inter-specific competition). We show that sexual plants are better inter-specific competitors than apomicts in terms of vegetative reproduction (number of stolons) and vegetative spread (stolon length), while apomicts do better than sexuals in intra-specific competition. The magnitude of the effect was in some cases dependent on the ploidy levels of the plants. Furthermore, apomicts always produced more stolons than sexuals, suggesting potential displacement of sexuals by apomicts where they co-occur.

Key words: apomixis; sexuality; inter-specific competition; intra-specific competition; invasiveness

INTRODUCTION

Apomixis, defined as asexual reproduction through seeds, is reported in over 400 species from more than 40 plant families {Asker:1992tz}. Apomixis is divided into sporophytic (embryos develop from sporophytic tissue) and gametophytic apomixis (embryos develop from gametophytic tissue), the latter being further subdivided into apospory and diplospory, depending on the cell type which develops into the female gametophyte {Koltunow:2003ie}. Both gametophytic types can be autonomous (neither fertilization of central cell nor egg cell to form the endosperm and embryo, respectively) or pseudogamous (fertilization of the central cell, but not of the egg cell). All types of gametophytic apomixis are facultative with varying levels of sexuality {Asker:1992tz}. Frequently, apomictic and sexual lineages co-occur in apomictic species {eg. *Taraxacum* and *Chondrilla* species,, vanDijk:2003wc}{*Hieracium pilosella*,, Mraz:2008ks}{*Ranunculus* species,, Horandl:2007uw}, with apomictic lineages being more widespread than sexual ones {Mraz:2008ks, Horandl:2006fb, Horandl:2007uw, vanDijk:2003wc}.

Because apomictic plants can produce offspring through seeds without the need of finding mates {no cost of sex,, Smith:1978td}, they are thought to have an advantage in colonizing habitats after disturbances and as pioneer plants in succession {Tomlinson:1966ub}. The colonizing ability of apomicts {Baker:1967vx}{Smith:1978td} is thought to be advantageous in invasive processes {Catford:2009ks}{Baker:1967vx}. Indeed, apomictic lineages are found to be among the first to invade new areas {eg. Krahulcova:2011jv}. Based on these arguments, apomicts are often considered to be more successful than sexuals.

However, most types of apomixis result in maternal clonal offspring (apospory, mitotic diplospory) {Asker:1992tz} and may therefore accumulate deleterious mutations over many generations {Muller's ratchet,, Muller:1964wr, Stebbins:1950tl, Stebbins:1957wr}.

Furthermore, apomictic populations are expected to have very low genetic variability, which drastically reduces their evolutionary potential to adapt to environmental changes. Sexual populations, in contrast, are expected to have high genetic variability and can, therefore, more easily adapt to environmental changes via evolutionary processes. Hence, the question arises whether the high abundance of apomicts is restricted to newly colonized habitats or if they may successfully co-exist with sexuals due to other advantages than those mentioned above.

Several hypotheses have been proposed in this context. For example, successful apomicts may contain general-purpose-genotypes {Baker:1965tj}{Lynch:1984wq}, avoid the cost of sex {Smith:1978td}, have a frozen niche variation that allows them to exploit heterogeneous habitats {Vrijenhoek:1979va, Vrijenhoek:1984wm}, or benefit from positive genetic diversity effects {cf. eg. Schmid:1994vk, Crutsinger:2006jt}. As mentioned above, all types of gametophytic apomixis have residual sexuality, even species which are referred to as obligate apomicts have some ($> 0\%$ but $\leq 2\%$) sexuality {Asker:1992tz}{Bicknell:2004fn}. Furthermore, meiotic diplospory can result in auto-segregation, as is the case in *Taraxacum officinale* {vanderHulst:2003ff}. In this type of apomixis, recombination but no reduction and fertilization occurs, leading to novel genetic configurations although the allelic composition is identical to that of the mother plant {Asker:1992tz}. In addition, since usually only the female gametophyte is affected by apomixis, populations of apomictic plants have a certain potential for adaptation via sexually produced genotypes. Indeed, van der Hulst and colleagues {*vanderHulst:2003ff} have found that the genetic variability in an apomictic population of *Taraxacum sp*. is as high as expected for a sexual species, which is mainly explained by hybridization with co-occurring sexual lineages and/or auto-segregation. Empirical data suggest that varying degrees of sexuality play the most important role for diversification in apomicts {Horandl:2007uw}.

Hieracium subgenus *Pilosella* is an example for an extremely successful invasive species complex including sexual and apomictic lineages. Sexual lineages of *H. pilosella* L. are obligate out-crossers and self-incompatible, although mentor effects (foreign pollen enables selfing) are described for this species {Krahulcova:1999ua}{Mraz:2003ds}. Apomictic lineages of *H. pilosella* are aposporous apomicts with autonomous endosperm development {Koltunow:1998ve}. Apomicts occur at ploidy levels from 4C to 8C {1C = haploid genome,, Greilhuber:2005ik, Mraz:2008ks} with varying levels of sexuality. Aposporous apomictic offspring are true maternal clones without chromosomal rearrangements, meaning that the offspring inherit the unchanged maternal genotype. Using *H. pilosella* as a model in ecological experiments thus avoids the confounding effects of auto-segregation and of ploidy effects. In addition, population maintenance in *H. pilosella* is possible by vegetative propagation via aboveground stolons for both sexual and apomictic lineages {Bishop:1978wf}. Using a grid-based simulation model, Winkler and Stöcklin {*Winkler:2002ww} have shown that most rosettes of *H. pilosella* are of vegetative origin, which enables sexual and apomictic lineages to persist over many growing seasons.

H. pilosella was accidentally introduced from Europe to New Zealand in the 1850s {Murphy:1878wu}, where, after a lag-phase, it became invasive and spread enormously since the 1950s {Scott:1990tp, Houliston:2004uy, Connor:1992wv}. During the lag-phase of invasion, hybridizations with another introduced apomict of the subgenus *Pilosella*, *Hieracium praealtum*, occurred at least 3 times and led to new genotypes {Trewick:2004wv, MorganRichards:2004dp}. It can be assumed that these genotypes were selected for traits promoting reproduction and spreading, such as apomictic seed production or a high vegetative reproduction rate via stolons. It has been shown that dense seed rain and high seedling survival largely contribute to the invasive success of *H. pilosella* in New Zealand {Makepeace:1985wu, reviewed in Day:2010hq}. Today, this species is particularly

widespread in disturbed short-tussock grassland dominated by *Festuca novae-zelandiae* {Makepeace:1985wu, reviewed in Day:2010hq}. The predominant cytotype occurring in New Zealand is an apomictic pentaploid {aP5,, apomictic *Pilosella* 5-ploid,, Chapman:2000tt}. In contrast, the main type occurring in Europe is a sexual tetraploid {sP4,, Mraz:2008ks}.

In the present study we compare the performance of apomictic lineages from New Zealand with sexual lineages from central Europe. We took apomictic plants from New Zealand for 2 reasons: First, we can assume that due to hybridizations and different selection regimes for the past 130 years, New Zealand and European lineages of *H. pilosella* diverged genetically from each other in terms of their population dynamics. Second, apomictic plants from New Zealand have a high level of apomixis {Bicknell:2003te, Houliston:2004uy}{Bicknell:2004fn}. We expected that the sexual lineages from Europe have been selected for competitiveness and persistence of populations and the apomictic lineages from New Zealand for high vegetative reproduction and spreading.

We carried out two experiments. First, in a common garden experiment, we compared offspring of *H. pilosella* plants from New Zealand with offspring of sexual populations from Europe for their intra-specific (within-species) and inter-specific (between-species) competitiveness. Second, we tested whether differences in competitiveness between sexual and apomictic lineages persisted when ploidy was equal. To do this, we compared the apomictic pentaploid lineages from New Zealand (aP5) with newly created sexual pentaploid lineages (sP5). Pentaploid sexuals were obtained by crossing of sexual hexaploid (sP6) and sexual tetraploid (sP4) plants. By comparing the two independent experiments, we can draw conclusions about the role of ploidy for the performance of *H. pilosella*.

In both experiments, fitness-related traits (biomass, number of stolons, length of the longest stolon) were measured and the effects of reproductive type and intra- vs. inter-specific competition were tested using mixed-effects models.

We found that the sexual lineages from European populations were more persistent in between-species competition against the grass *Bromus erectus* in terms of vegetative reproduction, irrespective of ploidy level or genetic background. However, when apomictic lineages from New Zealand grew in competition with sexual lineages from Europe (within-species competition) they had a superior fitness, suggesting that they can displace the sexual lineages in within-species competition.

MATERIALS AND METHODS

Plant Materials and Soil

Hieracium pilosella L. (syn. *Pilosella officinarum* Schultz & Schultz) is a self-incompatible perennial herbaceous species including sexual and apomictic lineages (autonomous apospory). Ploidy levels vary from 4C to 8C {1C = haploid genome,, Greilhuber:2005ik}{Mraz:2008ks} and apomictic and sexual lineages can occur at each ploidy level. Individual rosettes are monocarpic and die after flowering and production of aboveground stolons.

Sexual lineages were derived from two sexual tetraploid populations in the Czech Republic. One population was from Mšeno (M; N 50° 28′17.5′′, E 14° 38′1.2′′) the other from Jince (J; N 49° 46′45.7′′, E 13° 57′54.4′′). Seeds were kindly sent to Zürich by Anna Krahulcova (Academy of Sciences of the Czech Republic). Apomictic lineages were derived from an apomictic population in New Zealand at Hurunui River near Lake Sumner (S 42° 42', E 172° 08'). Seeds were kindly collected by Ross A. Bicknell (Plant & Food Research, New Zealand) in March 2009. Apomictic plants used in the experiments were grown from seeds of a seed pool consisting of seeds from several individuals from the New Zealand population. Since apomixis is facultative in this species (Sailer et. al., unpublished), and since seeds were collected from different individuals, we can assume that the seeds from NZ are a mixture of

several different apomictic pentaploid lineages (aP5). Currently, we do not have a reliable genotyping system to identify individual clones, so we could not test how many different apomictic lineages were used.

Sexual plants were derived from vegetatively propagated plants from the two Czech populations (M; J), and are a mixture of 3 sexual tetraploid lineages (sP4). To generate pentaploid sexual lineages (sP5), a sexual tetraploid (sP4, "J2") and several sexual hexaploid lineages (sP6) from the Morteratsch glacier foreland, Upper Engadin, Switzerland (GPS: 791859, 145592, Swiss Grid) were crossed. Each individual offspring from these crosses is a new genotype. The mixture of these genotypes was used in the P5 experiment.

After surface sterilization, seeds were germinated in petri dishes on half strength MS-medium {Murashige:1962wn} (containing MS salts (Carolina, Burlington, North Carolina), Sucrose (Applichem, Darmstadt, Germany) and Phytoagar (Gibco BRL, Paisley, Scotland)) in a Percival Scientific climatic cabinet (CU-36L6/D, CLF Plant Climatics GmbH**,** Wertingen, Germany) at 22°C/18°C (day/night) 14h light and 10h dark cycle. Seedlings were transferred to soil when they had produced two to three true leaves. Seedlings were grown in the greenhouse for three days under a humidor after being transferred to soil, and then put into the common garden or left in the greenhouse. We used a nutrient-poor soil ("Dachgartenerde extensiv", Ricoter Erdaufbereitungs AG, Frauenfeld, Switzerland) to mimic the field situation.

Experimental Design

In two independent experiments, "garden" and "P5", apomictic and sexual plants, were compared. Apomictic and sexual plants were subjected to 2 factorially crossed competition regimes, resulting in 4 different treatments: 1) no competition (Figure 1A), 2) within-species competition (neighbor of other reproductive mode, Figure 1B), 3) between-

species competition (presence of grass, Figure 1C), between- and within-species competition (Figure 1D). For both experiments, we used a randomized block design with blocks (replicates) including the different treatment combinations.

Plants were grown in plastic boxes (Georg Utz AG, Bremgarten, Switzerland) of 40 x 30 x 30 cm (L x W x D). The bottom of the boxes had holes and was covered with a 2-cm thick drainage mat to prevent root rotting in standing water. The boxes were covered with mosquito net (Windhager AG, Baar, Switzerland) cages to prevent pollination between boxes in the common garden.

There are two caveats to this design. First, in the within-species treatments apomictic and sexual Hieracium plants occur together in the same box, but twice per block (Figure 1B and 1D) whereas in between species completion apomictic and sexual Hieracium plants occur separately in a single box. Because we found no autocorrelation between residuals of co-occurring apomictic and sexual plants within boxes we used measurements of both types of plants as if they were grown independently in different boxes in the analysis. Second, the between-species competition regime (Figure 1C and 1D) followed an additive rather than substitutive design which maintains constant density for the target species (in our case *H. pilosella*) but increases the total density, since grass is added as a competitor and is not replacing Hieracium plants, as in the within-species treatments, where a substitutive design was used (Harper 1977).

In the garden experiment apomictic and sexual plants of *H. pilosella* were derived from field sites and were of different ploidy level. This experiment was performed in the common garden with several apomictic lineages from New Zealand and three sexual lineages from Europe, and included 5 replicates per treatment combination.

In the P5 experiment, apomictic lineages from the New Zealand population were used together with the created sexual pentaploid lineages to ensure that apomictic and sexual plants

were of the same ploidy level. Both apomictic and sexual plants were grown from seeds. This experiment was performed in the greenhouse and treatment combinations were replicated twice.

Harvest and Size Measurements

The garden experiment was carried out from May 2009 to September 2009 in the common garden of the Institute of Plant Biology of the University of Zürich, Switzerland. This experiment was harvested non-destructively, since we intended it to be prolonged for another growing season. Since we found clear effects after one season, we terminated this experiment. For harvesting biomass, all leaf material from rosettes and stolons that exceeded a length of 5 cm was cut with scissors. Stolons growing out of the limits of the boxes were cut off and collected separately. Stolon number was counted and the length of the longest stolon of each plant was measured as a proxy for maximum vegetative spread. For measuring vegetative spread, stolons were not stretched out to be measured, but remained in their positions in the boxes.

The P5 experiment was carried out in the greenhouse of the Institute of Plant Biology of the University of Zürich in Zürich, Switzerland in winter 2010/2011. At the end of the experiment, entire plants without roots were harvested and their biomass was measured. Stolons were counted and stretched out to measure their length. To measure biomass, harvested biomass was oven-dried for 48 hours at 80 °C and weighed to the nearest 0.1 g.

Ploidy Analysis

The ploidy level of experimental plants was controlled by ploidy analysis following the two-step method described by Dolezel and colleagues {*Dolezel:2007hq} with minor modifications. Leaf material from stolons was chopped with a razor blade in 5 cm diameter

petri dishes in 500 µL of 0.1 M citric acid (Fluka, Buchs, Switzerland), 0.5% Triton X-100 (Sigma-Aldrich, Steinheim, Germany). The solution was filtered through 30 µm filters (CellTricsTM, Partec, Görlitz, Germany) into 1.5 mL Eppendorf tubes (Sarstedt, Numbrecht, Germany). Nuclei were collected by centrifugation at 200 g for 5 min at room temperature (Centrifuge 5415D, Eppendorf, Schönebuch, Switzerland). The supernatant was removed and nuclei were resuspended in 40 µL 0.1 M citric acid, 0.5% Triton X-100. One hundred and sixty µL of staining solution (0.4 M Na_2HPO_4 (Merck, Darmstadt, Germany), 5.5 µg/mL DAPI (4`,6-diamidino-2-phenylindole, Invitrogen, Eugene, Oregon, USA), 0.2 µL/mL 2-mercaptoethanol (Sigma-Aldrich, Steinheim, Germany)) were added 2 min prior to analysis by the flow cytometer robotics (Quanta SC MPL, Beckman-Coulter, Nyon, Switzerland).

Statistical Analyses

Separate statistical analyses were carried out for each measured trait and the two experiments. We used linear mixed models with "Box" and "Block (Replicate)" as random factors and summarized results in Analysis of Variance (ANOVA) tables. The factor "Grass" compares boxes C and D, which contain grass, with boxes A and B, in which no grass is present (figure 1). The factor "Neighbor" in the analysis compares boxes B and D, in which neighboring plants are of the different reproductive mode, with boxes A and C, in which the neighboring plants are of the same reproductive mode (figure 1). The factor "Reproduction" compares apomictic with sexual plants. Biomass had to be ln-transformed to normalize residuals. Stolon number and stolon length could be analyzed without transformation.

First, an overall analysis of the complete dataset was performed. A triple interaction between "Grass", "Neighbor" and "Reproduction" was never observed. Therefore, the model was restricted to interactions between "Grass" or "Neighbor" and "Reproduction". These

interactions are differences in the response of apomicts and sexuals to the presence of grass or the different reproductive mode of neighboring plants.

Second, if a two-way interaction occurred, the dataset was split according to the interaction (no grass and presence of grass, or same neighbor and different neighbor; black and grey bars in figure 1 "Overall Analysis: Factors"), and apomicts and sexuals were compared separately within these two data subsets. This separation was made to facilitate the interpretation of interactions.

Third, an in-depth analysis was performed to test for differences between apomicts and sexuals in each of the 4 treatments (A, B, C, D in figure 1). In each of these 4 data subsets, apomicts and sexuals were compared.

In addition, in the garden experiment an analysis testing for differences between the different lineages used was performed. One sexual tetraploid (sP4) lineage, "J2", seemed to perform as well as the mixture of the apomictic pentaploid (aP5) ineages. Based on these results a data subset was selected which contained the mixture of aP5 lineages from New Zealand and the sP4 lineage "J2". This data-subset was then analyzed again as described above to test whether the observed effects were genotype-dependent or not.

Analyses were performed in R {RaLanguageandEn:2010wf} using the nlme package {nomeLinearandNon:Ld2PfMNj}, the ggplot2 package {ggplotelegantgra:tn} and the grid package {Murrell:2005vv}.

RESULTS

Growth

Biomass of Hieracium was always reduced by the presence of grass (treatments C + D compared to treatments A + B in figure 1) in the garden experiment ($F_{1,26} = 40.39$, $p < 0.001$, figure 2A, table 1). In the garden experiment, apomicts had generally a higher biomass than

sexuals ($F_{1,115} = 49.3$, $p < 0.001$, figure 2C, table 1). In-depth analysis revealed that apomicts are 2.08 times heavier than sexuals in between-species competition (C in figure 1; $p < 0.001$, table 2). No differences in growth of apomicts and sexuals were observed in the 3 other treatments.

In the P5 experiment, biomass of Hieracium was also reduced by the presence of grass ($F_{1,11} = 9.53$, $p = 0.010$, figure 2B, table 1). Furthermore, apomicts increased while sexuals decreased their biomass if the neighbor was of the different reproductive mode compared to the neighbor being the same (interaction: $F_{1,11} = 5.00$, $p = 0.047$, figure 2D, table 1; B + D compared to A + C in figure 1). This resulted in apomicts having a higher biomass if the neighbor is different ($t_{1,20} = 2.69$, $p = 0.014$, figure 2D, grey background). In-depth analysis revealed that apomicts were 3.14 times heavier than sexuals in within-species competition (B in figure 1; $p = 0.018$, table 2).

Vegetative Propagation – Number of Stolons

In the garden experiment, the presence of grass reduced the number of stolons compared to the treatment without grass as a competitor ($F_{1,32} = 70.8$, $p < 0.001$, figure 2E, table 1). Furthermore, the number of stolons was more strongly reduced in apomicts than in sexuals by the presence of grass (interaction: $F_{1,198} = 8.0$, $p = 0.005$, figure 2E, table 1). In the absence of grass, apomicts had more stolons than sexuals ($t_{1,99} = 5.0$, $p < 0.001$, figure 2E). In-depth analysis revealed that apomicts had 1.32 times more stolons than sexuals without competition ($p = 0.028$, table 2) and 1.33 times more stolons in within-species competition ($p < 0.001$, table 2).

The P5 experiment basically revealed the same effects as the garden experiment (figure 2F, table 1). However, in the in-depth analysis of the boxes without competition and of the boxes with both between- and within-species competition we found slightly different results: apomicts produced 2.01 or 1.06 times more stolons than sexuals under within-species

competition ($p = 0.049$, table 2) or under combined between- and within-species competition ($p = 0.019$, table 2), respectively.

Spread – Maximum Stolon Length (Longest Stolon)

In the garden experiment, presence of grass reduced the maximum stolon length compared to absence of grass ($F_{1,33} = 46.0$, $p < 0.001$, figure 2G, table 1). Furthermore, maximum stolon length was more strongly reduced in apomicts than in sexuals by the presence of grass (interaction: $F_{1,134} = 5.13$, $p = 0.025$, figure 2G, table 1). In the absence of grass, apomicts had a longer maximum stolon length than sexuals ($t = 2.7$, $p = 0.010$, figure 2G). In-depth analysis revealed that apomicts produced 1.14 times longer stolons than sexuals in the within-species treatment ($p = 0.034$, table 2).

In the P5 experiment, similar results as in the garden experiment were obtained (figure 2H, table 1, table 2).

Effects of Lineages (Genetic Background)

In the garden experiment a mixture of several apomictic lineages from New Zealand (see Materials and Methods) were compared to three sexual lineages from Europe. We found that the sP4 line "J2" had similar performance as the aP5 lineages. The results of the analysis comparing the sexual tetraploid "J2" to the pentapolid New Zealand lines are shown in figure 3. In general, the presence of grass influenced biomass of all lineages negatively ($F_{1,26} = 45.66$, $p < 0.001$, figure 3A), stolon count ($F_{1,34} = 52.56$, $p < 0.001$, figure 3B) and the length of the longest stolon ($F_{1,33} = 52.77$, $p < 0.001$, figure 3C). Furthermore, the number of stolons was more strongly reduced in apomictic New Zealand lines than in the sexual "J2" (interaction: $F_{1,118} = 5.56$, $p = 0.020$, figure 3B). In the absence of grass, New Zealand lines had more stolons than "J2" ($t = 2.34$, $p = 0.023$, figure 3B). This means that in terms of

vegetative propagation, sexual lines resist more to competition than apomictic ones, irrespective of their genotype.

DISCUSSION

We compared successful apomictic *H. pilosella* from New Zealand with sexual *H. pilosella* from Europe in order to determine whether the success of the New Zealand lineages is, at least in part, due to a fitness advantage in growth (biomass), vegetative propagation (stolon count), and spread (maximum stolon length). For all traits in both experiments, grass had a negative influence on the measured trait.

In the garden experiment, apomicts and sexuals differed in ploidy level while in the P5 experiment they did not. Comparing the results of both experiments therefore allows us to reason about the role of different ploidy level in competition. The general difference in biomass between apomicts and sexuals observed in the garden experiment, which is in concordance with a study in *Taraxacum sp.*, in which triploid apomictic plants had a higher biomass than diploid sexual plants {deKovel:2001wl}, was lost in the P5 experiment where ploidy of apomicts and sexuals was identical. In-depth analysis comparing results from the two experiments showed also differences in the effects of both types of competition and of stolon numbers among the two experiments, indicating ploidy effects. In other words, ploidy may differentially influence biomass and vegetative propagation depending on the type of competition.

In terms of vegetative propagation and spread sexual *H. pilosella* were less negatively affected by the presence of grass than were apomictic *H. pilosella*, irrespective of ploidy differences. From these results we conclude that apomictic populations have a lower between-species competitiveness than sexual populations and thus the latter would outperform the former in between-species competition in the longer run. In our experiments, we could not

observe that apomicts outcompeted sexuals as vegetative propagation was not different in the between-species competition regime, which might have been due to a too low competition pressure and the short duration of our experiments. Nevertheless, we would expect that sexual populations are more persistent over time in complex communities in the field than apomictic populations are.

In contrast to the between-species competition regime, apomicts always performed better than sexuals in the within-species competition regime, irrespective of ploidy differences. This might be due to the history of the lineages used in the experiments. In both experiments, aP5 plants were derived from a successful population in New Zealand, meaning that these lineages had undergone selection. The created sP5 lineages are all new genotypes which have not undergone selection. In principle, this is also true for the sP4 lineages derived from a wild population used in the garden experiment, since sexual lineages are obligate outcrossers. This means that seedlings derived from a crossing event are new genotypes. Even though the parents of the sP5 lineages and the sP4 lineages have successfully passed selection, that is, they have a beneficial allele combination, their offspring must not necessarily be successful as well (hidden cost of sex or segregation load). In other words, apomicts might have performed better in the within-species competition treatment because the sexual lineages used had disrupted beneficial allele combinations {destabilizing hybridization hypothesis,, Lynch:1984vv}. A third possible explanation is a genetic association of enhanced growth, vegetative reproduction and spread with apomixis. This would need testing in further experiments.

Apomicts perform better if they compete only with sexuals. But sexuals are less affected by competition from other species. These two results support the hypothesis that apomictic plants are pioneers that are replaced by sexuals in course of succession {geographic parthenogenesis,, Tomlinson:1966vu, Mraz:2008ks}{vanDijk:2000wc, Horandl:2009hr,

Cosendai:2010he}. Succession, for example after glacial retreat, would result in a distribution pattern with apomicts being more abundant in the early, pioneering stages of succession, and with sexuals being more abundant in later stages of succession, in which the community is more complex and can therefore vary in different geographic regions. Indeed, a cytogeographical study performed by Mraz {*Mraz:2008ks} revealed such a distribution pattern across Europe. Apomictic cytotypes (pentaploids) are found in Northern Europe in areas which were covered by glaciers during the latest ice age in Europe while the sexual cytotypes (tetraploids) are mostly found in central Europe.

In addition to different competitive abilities of apomicts and sexuals, this cytogeographical pattern could also be explained either by the general-purpose genotype hypothesis or the destabilizing hybridization hypothesis {Lynch:1984wq}. The general-purpose genotype hypothesis, which was first formulated by Baker {Baker:1965tj}, states that selection might favor genotypes which are adapted to a wide variety of environments. In other words, an apomictic general-purpose genotype will be able to colonize a recently deglaciated area such as Northern Europe very effectively. Sexuals however, will not be able to spread to Northern Europe since their fitness will be inferior to the fitness of apomicts, which are general-purpose genotypes {Lynch:1984vv}. Furthermore, successful sexual and apomictic genotypes will cross in areas in which they co-occur, leading to hybrids which have an inferior fitness, since the beneficial allele combination of their parents is disrupted {Lynch:1984vv}. Our results with the sexual lineages are in concordance with the latter hypothesis.

Another possible explanation for the cytogeographical pattern is that the niches occupied by apomicts did not change since the glaciers retreated {frozen-niche variation hypothesis,, Vrijenhoek:1979va, Vrijenhoek:1984wm}. Different to the general-purpose

genotypes, which occupy a wider ecological niche, different apomictic genotypes are occupying small niches, which in total span a wider niche than their sexual parents do.

The frozen-niche variation hypothesis implies that genetic diversity between apomictic populations should be greater than within populations, since we would expect that the niches differ with geographical distance and therefore, between populations. In New Zealand, in which the main cytotype is a P5 (Chapman et al., 2000), repeated hybridization of *H. pilosella* with *H. praealtum* resulted in genetic variation, enabling the invasion of different niches. Chapman et al. (2000) have shown that the genetic diversity within apomictic populations in New Zealand is low, but also that there is no correlation between geographical and genetic distance. They report that genetic variation between populations is higher than within populations, but the variation is low also between populations. We interpret this finding as genetic variation between general-purpose genotypes, and that the observed pattern cannot be explained with the frozen-niche variationn hypothesis.

Better vegetative reproduction and better vegetative spread could be attributes of a general-purpose genotype, which could then be fixed by apomixis. Thus, our's and Chapman's findings can be interpreted as supporting the general-purpose genotype hypothesis.

Conclusion

In this study, we showed that apomixis and — independently of the mode of reproduction — the ploidy level can affect the fitness of *H. pilosella*. Up to now we cannot distinguish whether the observed advantage of the New Zealand apomicts is solely due to the mode of reproduction, or if and to which extent it is due to the different genetic background of the plants from New Zealand. Clearly, our results suggest that the success of the invasive New Zealand lineages is due to a fitness advantage in growth and vegetative propagation

compared to the sexual lineages as they are mostly found in Europe. Interestingly, sexual plants were found to be less influenced by between-species competition, indicating that apomictic *H. pilosella* are favored in pioneer habitats but might be displaced with ongoing succession by sexual plants. More apomictic and sexual lineages will have to be analyzed to fully disentangle the effect of the genetic background from the effect of the mode of reproduction.

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References

TABLES

Table 1: ANOVA tables of the overall analysis using mixed effects models.

Significant effects are printed in bold. Interactions between "Reproduction" and "Grass" or "Neighbor" were found for each trait in both experiments, except for biomass in the Garden experiment. There, only significant main effects were found.

num DF – degrees of freedom of tested term (nominator), den DF – degrees of freedom of error term (denominator) * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$

Garden	ln(Biomass)				Stolon Count				Longest Stolon			
	num DF	den DF	F-value	p-value	num DF	den DF	F-value	p-value	num DF	den DF	F-value	p-value
(Intercept)	1	115	1689.49	<0.001***	1	198	484.81	<0.001***	1	134	1463.96	<0.001***
Grass	1	26	40.39	**<0.001*** **	1	32	70.75	**<0.001*** **	1	31	46.36	**<0.001*** **
Neighbor	1	26	0.00	0.968	1	32	1.03	0.317	1	31	2.11	0.157
Reproduction	1	115	49.29	**<0.001*** **	1	198	14.32	**<0.001*** **	1	134	3.58	0.061
Grass : Reproduction	1	115	0.15	0.695	1	198	7.97	**0.005** **	1	134	5.12	**0.025***
Neighbor : Reproduction	1	26	2.35	0.138	1	32	0.03	0.861	1	31	0.38	0.540

P5	ln(Biomass)				Stolon Count				Longest Stolon			
	num DF	den DF	F-value	p-value	num DF	den DF	F-value	p-value	num DF	den DF	F-value	p-value
(Intercept)	1	43	2555.39	<0.001***	1	27	162.74	<0.001***	1	27	366.14	<0.001***
Grass	1	11	9.53	**0.010***	1	11	11.78	**0.006** **	1	11	11.17	**0.007** **
Neighbor	1	11	0.01	0.925	1	11	1.61	0.231	1	11	2.28	0.159
Reproduction	1	43	5.01	**0.030***	1	27	8.32	**0.008** **	1	27	13.20	**0.001** **
Grass : Reproduction	1	43	0.09	0.763	1	27	5.77	**0.024***	1	27	5.29	**0.029***
Neighbor : Reproduction	1	11	5.00	**0.047***	1	11	0.05	0.834	1	11	2.32	0.156

Table 2: Differences in biomass, stolon count and stolon length (means, CV) between apomicts and sexuals in different treatments of the experiments according to the in-depth analysis. (Note that in the within-species competition treatment apomicts were always performing better, except for biomass in the garden experiment, see text for details).

* – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$

Experiment	Trait	Treatment	Apomict (CV)	Sexual (CV)	p-value
Garden	biomass [mg]	between-species	851 (0.44)	349 (1.05)	< 0.001***
	stolon count	none	7.5 (0.33)	5.6 (0.29)	0.028*
		within-species	7.6 (0.29)	5.7 (0.32)	< 0.001***
	longest stolon [mm]	within-species	477 (0.22)	415 (0.27)	0.034*

Experiment	Trait	Treatment	Apomict (CV)	Sexual (CV)	p-value
P5	biomass [mg]	within-species	3105 (0.59)	989 (0.59)	0.018*
	stolon count	within-species	8.6 (0.41)	4.4 (0.34)	0.049*
		between- & within-species	4.6 (0.64)	4.3 (0.37)	0.019*
	longest stolon [mm]	within-species	343 (0.22)	182 (0.27)	0.001**

FIGURE LEGENDS

Figure 1: Design and analysis of competition experiments with apomictic and sexual Hieracium pilosella. A, B, C, D rows are the 4 different competition treatments: A) no competition, B) within-species competition, C) between-species competition, and D) within- and between-species competition. The experimental unit are boxes (grey rectancles) with 6 and 4 plants for the garden (left) and P5 (right) experiment, respectively. Note that in B and D (within-species competition treatments) the apomictic (red circles) and sexual (yellow circles) plants are in different boxes. The density of Hieracium is always equal, but in the between-species competition treatments total density in boxes is increased due to the addition of grass as a competitor.

In the overall analysis, the factor "Grass" compares treatments A+B with treatments C+D, whereas the factor "Neighbor" compares treatments A+C with treatments B+D, as is indicated on the right side of the figure. For correct comparison of apomictic and sexual plants in case of interactions, the groups were split into no treatment (black bars) and treatment (grey bars). For the in depth analysis apomictic and sexual plants were compared for each treatment (A, B, C, D) separately.

Grey rectangles – boxes; yellow circle – sexual plant; red circle – apomictic plant; green circle – grass; sP4 – sexual Pilosella 4-ploid; sP5 – sexual Pilosella 5-ploidy; aP5 – apomictic Pilosella 5-ploid

Figure 2: General competition effects and reactions of apomictic and sexual Hieracium pilosella to different competition treatments on biomass, stolon number and stolon length in two experiments. **A, C, E, G)** Garden experiment **B, D, F, H)** P5 experiment.

There is a general reduction of biomass due to the presence of grass both in the garden **(A)** and in the P5 experiment **(B). C)** Apomicts have generally a higher biomass than sexuals.

D) Apomicts increase in biomass when the neighbor is a sexual, the latter decreasing in biomass. Apomicts have a higher biomass than sexuals when the neighbor is of different reproductive mode. Overall, the biomass increases when the neighbors differ in their reproductive mode. The presence of grass reduces the number of stolons in both the garden **(E)** and the P5 experiment **(F)**. Apomicts produce more stolons than sexuals in the absence of grass and sexuals show a more stable production of stolons in both the garden **(E)** and the P5 experiment **(F)**. The presence of grass reduces the maximum stolon length in both the garden **(G)** and the P5 experiment **(H)**. Apomicts have a longer maximum stolon length than sexuals in the absence of grass, and sexuals are more stable in their stolon length in both the garden **(G)** and the P5 experiment **(H)**.

Dots represent the mean and errorbars are ± 1 sem of the mixed-effects model. Grey backgrounds are competition treatments, except for C) where the grey background represents sexuals. n.s. – not significant, * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$

Figure 3: Effects and different reactions of apomictic pentaploid New Zealand lines and a sexual tetraploid line “J2” to different competition treatments.

A) There is a general reduction of biomass due to the presence of grass. **B)** The presence of grass reduces the number of stolons. New Zealand lines produce more stolons than line “J2” in the absence of grass. “J2” shows a more stable production of stolons. **C)** There is a general reduction of the maximum stolon length due to the presence of grass.

Dots represent the mean and error bars are ± 1 sem of the mixed effects model. Grey backgrounds are competition treatments. n.s. – not significant, * – $p < 0.05$, ** – $p < 0.01$, *** – $p < 0.001$

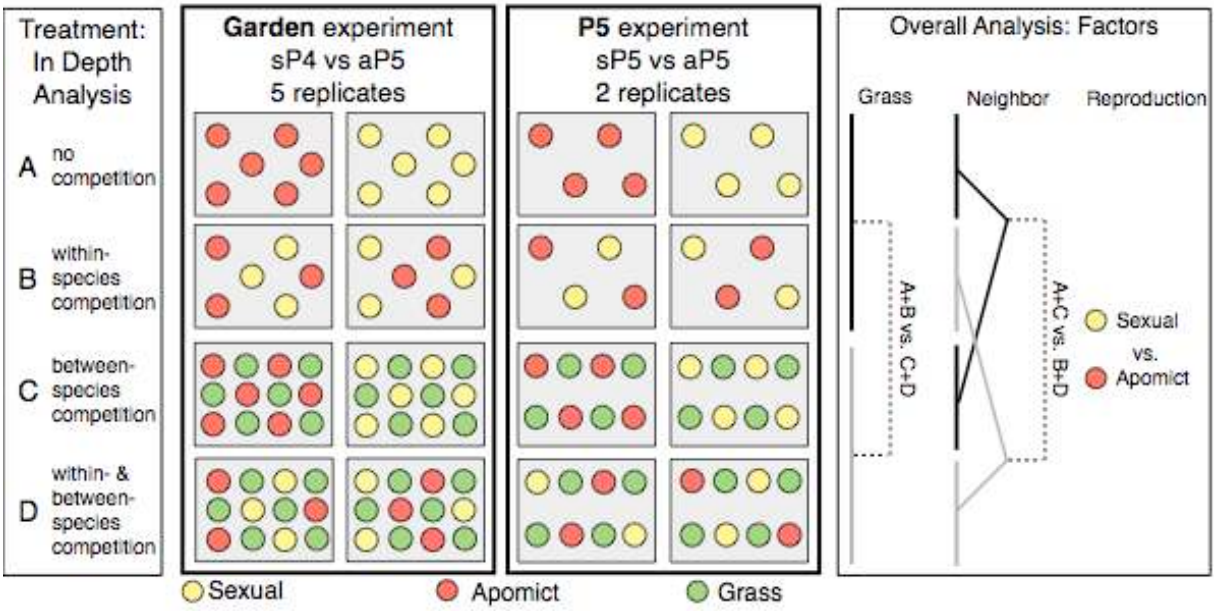
Treatment:
In Depth
Analysis
A no competition
B within-species competition
C between-species competition
D within- & between-species competition
Garden experiment
sP4 vs aP5
5 replicates
P5 experiment
sP5 vs aP5
2 replicates
Sexual
Apomict
Grass
Overall Analysis: Factors
Grass
Neighbor
Reproduction
A+B vs. C+D
A+C vs. B+D
Sexual
vs.
Apomict

Figure 1

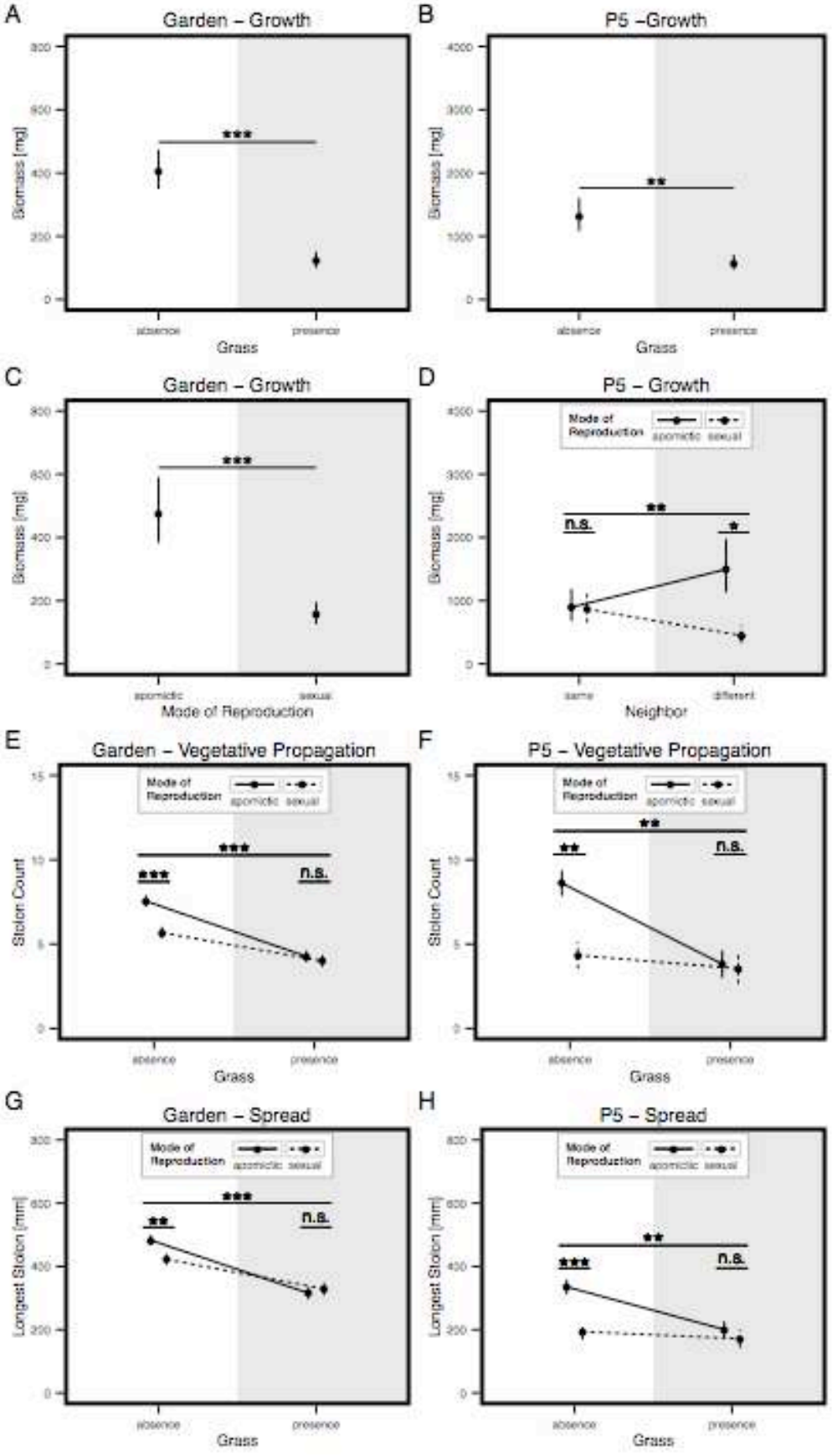
A
Garden – Growth
Biomass [mg]
***
absence
presence
Grass
B
P5 –Growth
Biomass [mg]
**
absence
presence
Grass
C
Garden – Growth
Biomass [mg]
***
apomictic
sexual
Mode of Reproduction
D
P5 – Growth
Mode of Reproduction
apomictic
sexual
Biomass [mg]
**
n.s.
*
same
different
Neighbor
E
Garden – Vegetative Propagation
Mode of Reproduction
apomictic
sexual
Stolon Count
***
***
n.s.
absence
presence
Grass
F
P5 – Vegetative Propagation
Mode of Reproduction
apomictic
sexual
Stolon Count
**
**
n.s.
absence
presence
Grass
G
Garden – Spread
Mode of Reproduction
apomictic
sexual
Longest Stolon [mm]
***
**
n.s.
absence
presence
Grass
H
P5 – Spread
Mode of Reproduction
apomictic
sexual
Longest Stolon [mm]
**
***
n.s.
absence
presence
Grass

Figure 2

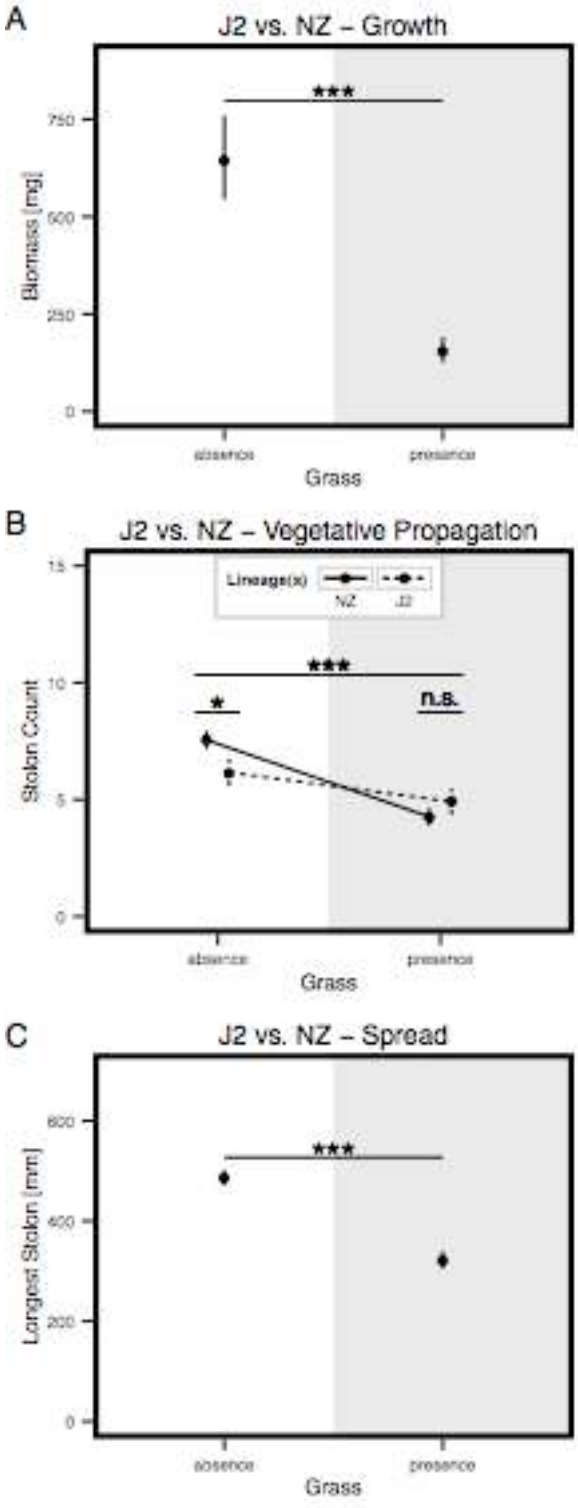
A
J2 vs. NZ – Growth
***
750
500
250
0
Biomass [mg]
absence
presence
Grass
B
J2 vs. NZ – Vegetative Propagation
Lineage(s)
NZ
J2
***
*
n.s.
15
10
5
0
Stolon Count
absence
presence
Grass
C
J2 vs. NZ – Spread
***
600
400
200
0
Longest Stolon [mm]
absence
presence
Grass

Figure 3